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PREPARATION OF CARBOBENZOXY-L-TYROSINE METHYL AND ETHYL ESTERS AND OF THE CORRESPONDING CARBOBENZOXY HYDRAZIDES

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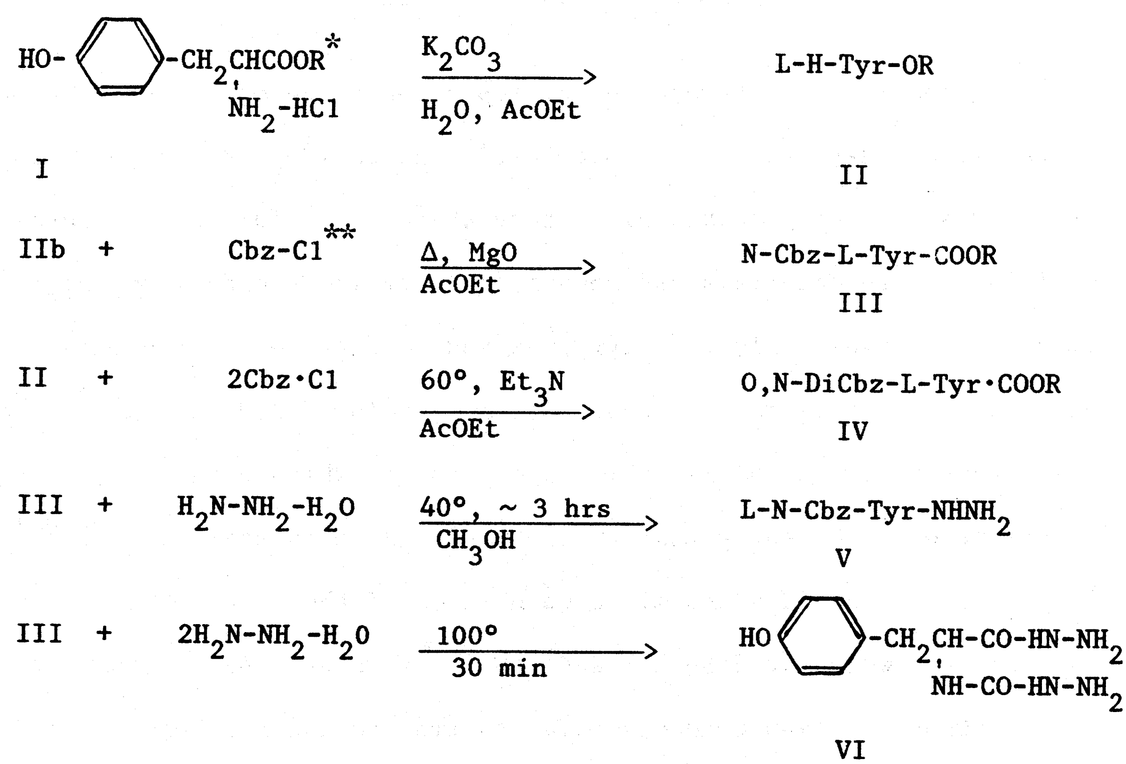
It appeared of interest to develop an easy and economical procedure for synthesis of heat-stable enterotoxins.[1] The solid-phase method[2] has been criticized[3] as being unsuitable for syntheses of acid-labile natural products, yielding impure materials and because of very low yields with polyfunctional amino acids, especially with tyrosine and asparagine. Since the carbodiimide procedure causes racemization and intramolecular dehydration,[4-7] attention was directed toward the reliable azide method[8] which gives little or no racemization.[8] Recently that method was used by several authors[10-12] to prepare small peptides and micro quantities of the enterotoxin. Since the carbobenzoxy amino acid methyl and ethyl esters are the key compounds for the azide method,[8] it appeared essential to improve the syntheses of this type of compounds and supplement their characteristics with some spectral data.

This paper describes the preparation of L-tyrosine methyl and ethyl esters (II), N-monocarbobenzoxy-L-tyrosine ethyl ester (III), O,N-dicarbobenzoxy-L-tyrosine methyl and ethyl esters (IVa and IVb), and the hydrazides (V and VI). On treatment with sodium nitrite, the monohydrazides produce azides.[8]

In general, the methods for the preparation of the carbobenzoxy esters[10,13,14] are not specific and are difficult to duplicate. Thus, for

III two different melting points, 92-94° and 78° are cited, and no data are given about the yields. The esters IVa and IVb have only been isolated as by-products.[10,13] While esters react readily with hydrazine, to give hydrazide,[8] but without heating the reactions are too slow and the yields are rather low[15,16] at room temperature. On the other hand boiling the ester with hydrazine for 1 hr[13] leads to the formation of dihydrazide[17] VI and of hydantoin,[18] it is also known that boiling hydrazine may result in an explosion.[19]



*Ia, IIa, IVa, R = CH_3; Ib, IIb, IVb, R = CH_2CH_3; **Cbz-Cl = Benzyl chloroformate

A excellent yield of N-monocarbobenzoxy-L-tyrosyl hydrazide (V) was obtained by heating a saturated methanolic solution of III with hydrazine hydrate for ~3 hrs at 40°. The formation of dihydrazide (VI) was confirmed by heating III with a small excess of hydrazine hydrate for ~30 min at 100°. The methyl ester (IVa) reacted, about three times faster then the ethyl ester IVb. By treatment of IVa with hydrazine hydrate at 40°, the product began to separate in 2 hrs and the reaction was completed in ~10 hrs; reacting ethyl ester IVb under the same conditions, solid begins to separate in ~6 hrs and the reaction required ~30 hrs to be completed. In contrast to III the dicarbobenzoxy esters IVa and IVb did not react with hydrazine at room temperature overnight.

EXPERIMENTAL SECTION

L-Tyrosine, benzyl chlororformate, hydrazine hydrate and other common chemicals were obtained from commercial sources. L-Tyrosine ethyl ester hydrochloride was prepared as outlined by Dymicky et al.[20] Optical rotation was determined on Perkin-Elmer 141 polarimeter, using 10 cm standard cell, volume 5 ml. IR (KBr) were determined on Perkin-Elmer[21] 421 grating spectrophotometer. ^{13}C-NMR were determined at 100.40 Mhz with JEOL-GX 400 FT NMR spectrophotometer, which includes a 9.4 Tesla Oxford narrow bore (54 mm) magnet and a DEC LCI 11/23 computer system. Measurements were carried out at 22° ± 1° (temperature of the probe). The samples were spun at 15 Hz in 10.1 mm NMR tubes, using CD_3OD as the solvent and CD_3CN as the reference, to which were assigned values of 49.00 ppm and 117.39 respectively. A 25,000 Hz spectral frequency range was examined with 35 K data points zero-field to 35K. Free induction decays were acquired with 16.0 μsec., 90° ^{13}C pulses, utilizing a pulse delay of 20.0 sec. A line-broadening factor of 1.0 Hz was applied. Proton decoupled ^{13}C spectra were obtained using single-pulse bilevel decoupling.

L-Tyrosine Ethyl Ester (IIb).- To Ethyl acetate (200 ml), water (150 ml) and 24.56 g (0.1 mole) of tyrosine ethyl ester hydrochloride (Ib) in a 500 ml separatory funnel, 8.0 g of potassium carbonate was gradually added, to pH 7.5. After each addition, the mixture was thoroughly shaken; at the end both layers became clear. The acetate layer was then separated, the aqueous layer was extracted twice with 50 ml ethyl acetate. The extracts were combined, dried over 30 g of anhydrous sodium sulfate and filtered. The solvent was distilled off under reduced pressure, the dry residue was recrystallized from benzene, 20 ml/g, whereupon 17.80 g (85%) of free ester (IIb) was obtained, mp. 102-103°, lit. mp. 108-109.5°; $[\alpha]_D^{25}$ = + 21.0° (c 2, AcOH), + 20.45

(c 4.85, EtOH), lit.[22] $[\alpha]_D^{20}$ = + 20.40 (c 4.85, EtOH). Identity was confirmed by IR, and ^{13}C-NMR, and purity by HPLC.

Anal. Calcd. for $C_{11}H_{15}NO_3$: C, 63.14; H, 7.22; N, 7.17

Found: C, 63.33; H, 6.95; N, 7.02

IR (KBr): 3690, 3280, 3220, 2300, 1710, 1560, 1475, 1425, 1225, 1140, 1020, 785 and 490 cm^{-1}.

^{13}C-NMR (CD_3OD): δ 14.89 (CH_3), 61.72 (CH_2, ester), 175.83 (CO, ester), 57.28 (α CH), 40.74 (β CH_2), 128.68 (C_1, ring), 157.33 (C_4, ring).

L-Tyrosine Methyl Ester (IIa).- L-Tyrosine, 18.10 g (0.1 mole) and 300 ml ~2.5 N. HCl in methanol were placed in a 500 ml flask equipped with a stirrer and condenser and immersed in a silicone bath heated at 70-75° and stirred overnight (~15 hrs). The solution was concentrated to dryness, under reduced pressure. The residue was dissolved in 200 ml methanol, a slight excess of triethylamine was added (as required to bind HCl), and stirred for 30 min at ~70°. The $Et_3N \cdot Cl$ salt was collected, and the filtrate was concentrated to dryness under reduced pressure. The dry residue was recrystallized from ethyl acetate, 15 ml/g, whereupon 15 g (77%) of crystalline product, mp. 129-131; was obtained. A second recrystallization from ethyl acetate raised the mp. to 135-136°, Lit.[22a] mp. 135-136°, $[\alpha]_D^{25}$ = + 25.90° (c 2, CH_3,OH); lit.[22a] $[\alpha]_D^{25}$ = + 25.75° (c 2, CH_3OH).

Anal. Calcd for $C_{10}H_{13}NO_3$: C, 61.52; H, 6.71; N, 7.17

Found: C, 61.38; H, 6.85; N, 7.05

N-Monocarbobenzoxy-L-tyrosine Ethyl Ester (III).

Method A. (Using one half of IIb to bind hydrogen chloride).- L-Tyrosine ethyl ester 20.92 g, (0.1 mole) and 300 ml ether were placed in a 500 ml three-neck reaction flask equipped with a condenser, stirrer and separatory funnel and immersed into a silicone bath at 40°. The mixture was stirred and 9.38 g (0.05 mole) of benzyl chloroformate in 100 ml ether was added dropwise.

Within ~1 hr the addition was completed and heating and stirring were continued for an additional 1 hr. The mixture was then filtered by suction, the residue was washed with 100 ml ether, the filtrates were combined and the residue was dried at 25°/0.1 mm, whereupon 11.85 g (~0.05 mole) of Ib was recovered. After distilling the solvent from the filtrate, 15.5 g of III (~0.05 mole) was isolated. This was dissolved in carbon tetrachloride (7 ml/g) and left at room temperature overnight. The crystallized product was filtered and dried at 25°/0.1 mm, whereupon 13.5 g of III (~46%) was obtained, mp. 77-77.5°, $/\alpha/_D^{25}$ = - 3.05° (c 2, EtOH). Lit.[14], mp. 78°, $/\alpha/_D^{25}$ = -4.70 (EtOH). Lit.[13], mp. 92-94°, $[\alpha]_D^{13}$ = -1.0 (c 1). Solvent is not given, yield 4.98%.

Anal. Caldc. for $C_{19}H_{21}NO_5$: C, 66.45; H, 6.16; N, 4.07

Found: C, 66.22; H, 5.98; N, 4.06

IR (KBr): 3260, 2260, 1685, 1635, 1615-1605 (a weak split), 1510-1470 (a weak triplet), 1425, 1285, 1180, 1125 and 705 cm^{-1}. ^{13}C-NMR (CD_3OD): δ14.89 (CH_3 ester), 62.06 (CH_2, ester), 173.44 (CO, ester), 57.76 (α CH), 49.01 (β CH_2), 128.42 (C_1, ring), 157.11 (CO, N-Cbz), 67.28 (CH_2, N-Cbz), 127.90 (C_1, ring, Cbz).

Method B. (Using magnesium oxide to bind hydrogen chloride).- About 500 ml ethyl acetate, 31.70 g (0.129 mole) of Ib and 10.40 g of magnesium oxide in a 1 L three-neck reaction flask equipped as given above, were stirred at 70°, and 24.22 g (0.142 mole) of 95% of benzyl chloroformate in 100 ml ethyl acetate was added dropwise. In about 2 hrs the additional was completed. The reaction mixture was refluxed for 1 hr, filtered by suction, and the residue was washed with 100 ml ethyl acetate. The filtrates were combined, and the solvent was distilled off under reduced pressure. The oil-like residue (~40 g) solidified by standing at room temperature overnight. This was dried at 25°/0.1 mm and recrystallized from carbon tetrachloride, 7 ml/g, giving 26.30 g (60%) of III, mp. 76-77.5°. The second recrystallization; 12 ml/g, mp. 78-79°, $/\alpha/_D^{25}$ = - 3.02° (c 2, EtOH). IR (KBr) and ^{13}C-NMR spectra as given above. Lit.[13,14]: mp. 92-94° and 78°, respectively.

<u>O,N-Dicarbobenzoxy-L-tyrosine Methyl Ester (IVa)</u>.- Into a 500 ml three-neck reaction flask, equipped as above (III, Method A), were placed 19.52 g (0.1 mole) of IIa, 400 ml ethyl acetate, and 20.20 g (0.22 mole) of triethylamine. The solution was stirred at 60°, and 37.52 g (0.22 mole) of benzyl chloroformate in 50 ml ethyl acetate was added dropwise. In 30 min the addition was completed, the temperature of the bath was raised to 70°, heating and stirring continued for an additional 1 hr. The mixture was filtered hot, the residue was washed with 100 ml of ethyl acetate and dried at 56°/0.1 mm, whereupon 26.48 g of triethylamine hydrochloride was obtained, 96%. The filtrates were combined, the solvent was distilled off under reduced pressure, and the residue was dried, giving 40.30 g of pale-pinkish product, ~87%. This was recrystallized from isopropanol, 12 ml/g, giving 31.28 g (77.6%) of the product, mp. 108-109°, $/\alpha/_D^{25}$ = - 31.10°, (c 1, DNF). Lit.[10]: mp. 110-111°, $/\alpha/_D^{23}$ = - 33.5°, (c 1, DMF), yield 4.20%.

<u>Anal</u>. Calcd. for $C_{26}H_{25}NO_7$: C, 67.37; H, 5.44; N, 3.02

Found: C, 67.63; H, 5.40; N, 2.99

IR (KBr): Identical, as given below for IVb.

<u>O,N-Dicarbobenzoxy-L-tyrosyl Ethyl Ester (IVb)</u>.- This compund was prepared from IIb as described above for preparation of IVa, whereupon 92% of solid residue was obtained, which was recrystallized from isopropanol, giving 66% of the final products, IVb, mp. 99-100°, $/\alpha/_D^{25}$ = - 9.20° (c 0.25, EtOH); -26.50° (c 1, DMF); + 9.15 (c 2, AcOH). Lit.[13]: mp. 104°, $/\alpha/_D^{13}$ = - 2.0° (c 1). Solvent is not given, yield 7.0%.

<u>Anal</u>. Calcd. for $C_{27}H_{27}NO_7$: C, 67.91; H, 5.70; N, 2.93

Found: C, 68.10; H, 5.77; N, 3.04

IR (KBr): 3357, 1746, 1731, 1690.7, 1532. 1266.3, 1218. 1178, 1059, 744 and 706.3 cm^{-1}. ^{13}C-NMR (CD_3CN): δ14.32 (CH_3, ester), 62.03 (CH_2, ester), 172.40 (CO, ester), 56.31 (α CH), 37.42 (β CH_2), 128.50 (C_1, ring, Tyr), 66.98 (CH_2, N-Cbz), 70.32 (CH_2, O-Cbz), 150.96 (CO, N-Cbz), 154.40 (CO, O-Cbz).

N-Carbobenzoxy-L-tyrosyl Hydrazide (V).- Into a 50 ml Erlenmeyer flask 3.40 g (0.01 mole) of III and 5 ml methanol were placed, slightly heated until dissolved, stirred and 2 ml (~0.04 mole) of hydrazine hydrate were added. The flask was equipped with a condenser, immersed into a silicone bath at 40-45° and heated for 1 hr, then stored at room temperature for a few hours and filtered by suction. The residue was recrystallized from ethanol, 45 ml/g, giving 2.87 g (84%) of the product, mp. 222-224°, $/\alpha/_D^{25}$ = + 11.40° (c 1, NaOH). Lit.[16,23]: mp. 220°. No data on optical rotation.

Anal. Caldc. for $C_{17}H_{19}N_3O_4$: C, 61.99; H, 5.81; N, 12.75

Found: C, 61.78; H, 6.05; N, 12.48

IR (KBr): 3298, 3269, 1690, 1666, 1626, 1535, 1514, 1270, 1250, 1172, 1042, 738, and 542 cm^{-1}.

Remark: Using the procedure given in the literature[13,23], a mixture was obtained consisting predominatly of mono and dihydrazides. The procedure cited in the reference[13] yielded dihydrazide as the main product.

L-Tyrosyl Dihydrazide (VI).- Into a 25 ml reaction flask 3.44 g (0.01 mole) of III and 2 ml of hydrazine monohydrate were placed and the mixture was heated for 30 min at 100°, simulating the procedure of Ishida and Onishi.[13] Solidified material was recrystallized from methanol, 42 ml/g, whereupon 1.84 g (73%) of crystalline product was obtained, mp. 181-183°, $/\alpha/_D^{23}$ = + 1.80° (c 1, EtOH). Lit.[17] mp. 185-186°. Optical rotation not given.

Anal. Calcd. for $C_{10}H_{15}N_5O_3$: C, 47.60; H, 5.99, N, 27.76

Found: C, 47.42; H, 5.90; N, 27.61

IR (KBr): 3290, 3220, 1630, 1530, 1500, 1435, 1390, 1350, 1310, 1290, 1230, 1160, 1130, 1090, 1050, 960 and 550 cm^{-1}.

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